Albumen & Sugar Testing

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ON THE

VARIOUS MODES OF TESTING

FOR

ALBUMEN AND SUGAR IN THE URINE

TWO LECTURES

BY

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LONDON
SMITH, ELDER, & CO., 15 WATERLOO PLACE
1884

PREFACE.

THE SUBSTANCE of the following Lectures has already been published. After careful revision they are now republished, with considerable additions, in the hope that, by facilitating the detection and estimation of albumen and sugar in the urine, the practice of testing for those products *in every case of disease* will be, in some degree, promoted.

11 SAVILE ROW:
April 1884.

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LECTURE I.

ON THE VARIOUS MODES OF TESTING FOR ALBUMEN IN THE URINE.

GENTLEMEN,—I propose to describe, demonstrate, and critically review the various modes of testing for albumen in the urine in the light of recent knowledge and research.

The two tests for albumen which have hitherto been most generally used are heat and nitric acid. I pour into a test-tube a column of urine about two inches in height, and place it over a spirit-lamp. Before the liquid reaches the boiling point, the albumen begins to coagulate; and the more abundant the albumen, the lower is the temperature at which coagulation commences. When the urine is highly albuminous, as the heating proceeds and coagulation commences, the tube must be continually and briskly shaken, to prevent the sudden expulsion of the partially coagulated liquid by the

steam generated beneath a dense film on the surface. Now observe that, when albuminous urine is alkaline, heat alone will not show the presence of albumen. I take some of this albuminous urine, and before applying heat, I add a drop or two of liquor potassæ. You see that, when boiled, it remains clear; the potash has combined with the albumen, and the resulting albuminate of potash is not coagulable by heat; but if now I add a few drops of strong nitric acid, coagulation at once takes place.

Again, some specimens of urine, when boiled, give a precipitate which has the appearance of albumen, though none is present. The non-albuminous urines which are thus acted on by heat are usually neutral or alkaline, and the turbidity is due to the precipitation of phosphates. To a specimen of normal urine I add a drop or two of liquor potassæ, and, when heated over the lamp, you see that it becomes turbid.

This phosphatic turbidity is easily distinguished from an albuminous coagulum by the addition of a drop or two of nitric acid, or even of acetic acid, or citric acid solution, which dissolves the phosphates while coagulated albumen remains undissolved.

You see, then, that if heat alone were relied upon as a test for albumen, it might mislead in two opposite directions: (1) by not detecting albumen in alkaline urine, and (2) by giving a delusive phosphatic precipitate in non-albuminous urine.

Nitric acid has long been known and employed as a valuable test for albumen. To this clear specimen of urine, I add a few drops of strong nitric acid, and an abundant coagulum of albumen is immediately formed. In most cases, nitric acid alone is a sufficient test for the presence of albumen in the urine, but there are some exceptional conditions, and some sources of fallacy, which I now proceed to point out.

Here is a specimen of albuminous urine, which, as you see, is highly turbid with urates. As I warm it over the lamp, it first becomes clear by the solution of the urates, and then, by the further application of heat, the albumen coagulates, and renders the urine milky. Such a specimen requires to be cleared by heat before nitric acid can be satisfactorily used as a test; although, as you see, a slight excess of nitric acid added to the turbid urine decomposes and dissolves the urates, and then precipitates the albumen.

It sometimes happens that, when nitric acid is added to highly acid urine, it causes a turbid deposit of urates, which might be mistaken for an albuminous coagulum. The distinction is readily made by the application of heat, which dissolves the urates, but not the albuminous precipitate.

In the urine of patients who are taking copaiba, nitric acid, acting on the resin, causes a slight milkiness, which has sometimes been mistaken for albumen. Such urines have a peculiar resinous odour; although acid, they are not coagulated by heat, nor, as I shall presently show you, by picric acid, which is a most delicate test for albumen.

When an excess of nitric acid is added to a highly concentrated urine, it sometimes happens that a copious crystallisation of nitrate of urea gradually occurs. This slowly formed crystalline deposit could scarcely be mistaken for an amorphous coagulum of albumen.

With regard to nitric acid as a test for albumen I have yet to show you certain facts which require attention. To this specimen of highly albuminous urine I add a single drop of strong nitric acid. You see that it forms a white coagulum which, on

shaking the tube, is redissolved; a second drop may be added with the same result, while the further addition of acid causes a permanent precipitate. Now, note this fact, that, when a drop or two of nitric acid has caused a coagulum which has been redissolved, the subsequent application of heat will not precipitate the albumen. The explanation is, that a nitrate of albumen has been formed, which is not coagulable by heat, but readily by an excess of the acid. Albumen is a neutral substance which is capable of combining, on the one hand, with alkalies, as we have before seen; and, on the other, with acids; and, in both instances, to form compounds not coagulable by heat.

As a drop or two of nitric acid will prevent the coagulation of albumen by heat, so a great excess of nitric acid added to the urine will either redissolve the coagulum which first forms, or, if rapidly poured in, entirely prevent coagulation, the mixture remaining quite clear.

Nitric acid, carefully added to cold urine, is one of the most delicate tests for a slight trace of albumen in the urine. For this purpose, a few drops of the acid may be placed at the bottom of a test-

tube, and the urine slowly poured into the sloping tube, so as to rest on the surface of the acid; or, the urine being first placed in the tube, a few drops of the heavy acid are allowed to fall through the urine to the bottom. The opalescent albuminous coagulum appears at the junction of the two liquids.

In *picric acid*, we have a more delicate test for minute traces of albumen than either heat or nitric acid, or than both these tests combined. Picric acid may be used in the form of a saturated aqueous solution, or in the form of crystals or powder. An ounce of water at 60° Fahr. retains in solution 5·3 grains of the dry acid. A saturated aqueous solution may, therefore, be made by dissolving the powder in boiling distilled or rain water, in the proportion of six or seven grains to the ounce. A portion of the acid will crystallise out on cooling, leaving a transparent yellow supernatant liquid.

I now show you that this solution, being added to an equal volume of urine, in a test-tube, immediately coagulates the albumen. The coagulum is made more dense by the subsequent application of heat. The coagulated picrate of albumen is solu-

ble in strong alkalies. If, therefore, the albuminous urine were highly alkaline, it might be necessary to acidulate it with acetic or citric acid, before adding the picric solution. The picric acid solution is itself sufficiently acid to dissolve the phosphatic sediment which results from boiling a neutral or alkaline specimen of urine.

When the albumen is very copious, a few drops of picric acid cause cloudiness, which disappears on shaking, like the cloudiness caused by a drop or two of nitric acid. A further addition of the solution causes a permanent coagulation. A small amount of picric acid solution does not prevent the subsequent coagulation of the albumen by heat, and an albuminous precipitate by picric acid is not soluble by an excess of the acid. In these two respects, picric acid differs advantageously from nitric acid.

To detect a very minute trace of albumen, I adopt the method which I will now show you. Into a test-tube six inches long I pour a four-inch column of urine; then, holding the tube in a slanting position, I gently pour an inch of the picric acid solution on the surface of the urine, where, in consequence of its low specific gravity (1005), it

mixes only with the upper layer of the urine; and, as far as the yellow colour of the picric solution extends, the coagulated albumen renders the liquid turbid, thus contrasting with the transparent unstained urine below. Bear in mind that, for the action of the test, there must be an actual *mixture*, and not a mere surface-contact, of the two liquids. When, in consequence of the scantiness of the albumen, the turbidity is very slight, the application of heat to the upper part of the turbid column is found to increase the turbidity. If, then, the tube be placed in a stand, the coagulated albumen will gradually subside, and, in the course of an hour or so, forms a delicate horizontal film at the junction of the coloured and the unstained stratum of urine; the yellow liquid above, and the unstained urine below, being quite free from turbidity. Any previous turbidity in the urine interferes with the detection of a minute trace of albumen. The turbidity which is due to lithates may be removed by heat, and that caused by mucus by a previous filtration of the urine to be tested. I have proved, by numerous observations, that picric acid, applied in this way, is a more delicate and trustworthy test than nitric acid added to cold urine.

The simplest method of comparing the two tests, as regards their relative delicacy, is to dilute a specimen of albuminous urine until one or the other test fails to act; and it will be found that the picric acid solution shows the presence of albumen in a specimen diluted much beyond the point at which the nitric acid fails to give any indication of its presence. The picric acid often gives an immediate albuminous opalescence in urine, when nitric acid only slowly, after an interval of some minutes, gives a similar, but perhaps a doubtful, indication. I shall presently have something to say on the practical value of a test for minute traces of albumen.

Another mode of detecting a minute trace of albumen is to add about $\frac{1}{3}$ grain of picric acid powder (as much as can be carried on the point of a penknife) to about a drachm of urine in a test-tube and boil. As the picric acid dissolves the urine becomes more or less turbid, in proportion to the amount of albumen. If no albumen is present a transparent yellow liquid results from the solution of the picric acid in the urine.

Here let me remark that the albuminous opalescence with picric acid, which always occurs im-

mediately, if at all, and which is increased by heat, may readily be distinguished from the coarse granular particles of urate of soda which, after a delay of some minutes, sometimes result from the acidity of the picric solution. The granular masses of urates, often mixed with crystals of free uric acid, quickly fall to the bottom of the test-tube, and carry with them so much of the picric colouring matter that, when placed under the microscope, they are so opaque as to appear almost black.

It very rarely happens that the picric solution causes an immediate turbidity in non-albuminous urine caused by the precipitation of urates. I have met with this result in only three or four specimens out of some hundreds. This turbidity, like the similar turbidity sometimes caused by nitric acid, is readily distinguished from albumen by its speedy and complete disappearance when heat is applied.

It has been objected to picric acid as a test that it gives with *peptones* a precipitate not distinguishable from albumen. To this statement, I reply, first, that the precipitate with peptones is most easily distinguished from that with albumen by its ready solubility when heated. I have here a peptonised solution of mutton-fibre; the addition

of picric acid solution causes a copious yellow precipitate, which, as you see, is entirely dissolved over the spirit-lamp before the liquid reaches the boiling point. The distinction, then, between picrate of albumen and picrate of peptone is sufficiently easy. In fact, *there is no known substance, occurring in either normal or abnormal urine, except albumen, which gives a precipitate with picric acid insoluble by the subsequent application of heat.*

Precipitated peptones resemble a sediment of urates in the fact that both are dissolved by heat, but they are readily distinguished by the microscope, which shows the urates to be composed of large granules of urate of soda and uric acid crystals. Freshly precipitated peptones appear under the microscope quite homogeneous and free from solid particles; but when, after being dissolved by heat, they are reprecipitated on cooling, they contain exceedingly minute granules, which are incessantly dancing about with the so-called 'Brownian movement.'

This granular condition occurs at once if the cooling be allowed to take place *slowly* in the air, but if the liquid be *suddenly* cooled, by plunging the test-tube in cold water, the precipitate does

not assume the granular appearance until it has been allowed to stand for some hours.

The test for peptones which hitherto has been chiefly relied upon is Fehling's copper solution, which gives a rose-red colour at the junction of the two liquids, when the urine is gently poured on the surface of the solution previously introduced into the test-tube. I have met with specimens of highly albuminous urine which have given a rose-red or violet colour with Fehling's solution ; but after the coagulation of the albumen by heat,[1] and its separation by filtration, the filtrate either gave no precipitate with picric acid or a slight cloudiness not dissolved by heat ; the cloudiness, therefore, was due to previously uncoagulated albumen and not to peptones. I conclude that Fehling's solution alone is not a trustworthy test for peptones. Out of some hundreds of specimens of urine which I have tested for peptones I have found them in only one specimen, which was kindly sent to me by Dr. Oliver. I conclude, therefore, that their occurrence in the urine is by no means frequent, for with the picric acid test, and the subsequent application of heat, it is scarcely possible that they

[1] Peptones are not coagulated by heat.

can escape detection. That picric acid is a much more delicate test for peptones than Fehling's solution is shown by the fact that the addition of artificially prepared peptones to normal urine, in amount sufficient to give a copious precipitate with picric acid soluble by heat, affords only a slight colour indication with Fehling's solution.

If peptones are associated with albumen in the same specimen, their detection and separation may be readily effected by the picric acid and heat tests. The precipitate with picric acid, instead of being increased and rendered more dense by heat, as when albumen alone is present, will be lessened and dissolved in proportion to the amount of peptones present. If the boiling liquid be then poured on a filter, the dissolved picrate of peptone will pass through and precipitate again on cooling, while the coagulated albumen remains on the filter.

Dr. J. Frank Nicholson, writing to the 'Lancet' (November 10th, 1883, p. 835), mentions the fact that, in the urine of patients taking large doses of quinine, he got with picric acid copious opalescence, which was cleared by heat. A similar precipitate occurs when cinchonidine is taken in large doses. In fact, most of the vegetable alkaloids,

such as morphia, atropine, &c., are precipitated by picric acid and by the potassio-mercuric iodide; but the cinchona alkaloids are alone likely to be taken in sufficient doses to render the urine opalescent with either of these tests, and then the complete clearance by heat at once distinguishes them from an albuminous precipitate. The precipitate with the vegetable alkaloids is seen by the microscope to be finely granular, and so unlike freshly precipitated peptones (see p. 11).

I have shown you that picric acid is a most delicate and trustworthy test for albumen in the urine; and if you ask whether there is any practical advantage to be gained by the employment of so sensitive a test, I reply that, unquestionably, there is. For instance, during the convalescence from acute nephritis with albuminuria, whether the result of scarlet fever, of exposure to cold, or of the other well-known exciting causes, it is of vital importance to be assured that the urine is entirely free from albumen before the patient is allowed to pass from medical supervision and to return to his usual habits. For the want of such exact and careful observation, it too often happens that, in these essentially curable cases, the recovery is in-

complete ; a trace of albumen remains, but unattended by any signs of functional disorder, until, it may be many years afterwards, the patient presents himself with some of the many distressing symptoms and unequivocal physical signs in the urine, the heart, the arteries, the eyes, and various other tissues, of advanced and incurable degeneration of the kidneys.

And here let me warn you to avoid the not uncommon mistake of *testing for albumen only a specimen of urine passed before breakfast.* It frequently happens that, while the urine which is passed after resting in bed and before breakfast is quite free from albumen, that which is secreted after food and exercise contains albumen in abundance. It is, therefore, obvious that, if a morning specimen alone were tested, a patient might be considered and pronounced convalescent while disease of a dangerous tendency is still present. It is always desirable to have for testing both a night and a morning sample of urine ; the former showing the effect of food, exercise, and change of temperature, the latter of abstinence, rest, and warmth.

It will very commonly be found that the amount of albumen in the urine secreted after food and

exercise is twice as great as that passed after some hours of abstinence and rest in bed.

The erect posture alone has sometimes a remarkable influence. In one of my patients lately, a youth recovering from acute albuminuria, it was found at one period that, although no albumen appeared after his breakfast, so long as he remained in bed, soon after he assumed the erect posture, whether before or after breakfast, although he remained in the same warm room, a trace of albumen was found in the urine. The only probable explanation of this is that the erect posture caused some congestion of the kidneys, and consequent escape of albumen by the gravitation of blood into their weakened and relaxed vessels.

There is yet another class of cases in the investigation of which it is of practical importance to employ a delicate and trustworthy test for albumen. I refer to that common form of Bright's disease in which the small red granular, or so-called cirrhotic, kidney is found after death. In this class of cases, the amount of albumen is often small, and is no index or measure of the gravity of the disease; indeed, more than one recent observer has asserted that an entire absence of albumen is

of common occurrence in this form of disease. This statement, however, is not in accordance with my experience. The cases of this disease that have come under my observation have been very numerous, and I am constantly meeting with fresh examples; and when the existence of the disease has been unequivocally established by other signs and symptoms, I have very rarely found that a careful testing of the urine at different periods of the day has failed to detect albumen. The idea that the urine is often free from albumen in these cases must, I think, have resulted from carelessness or want of skill in testing.

One method of testing which is in common use is very likely to mislead; I refer to the practice of adding acetic acid to the urine before boiling. The result often is that, albumen not being coagulable by an excess of the acid, an acetate of albumen is formed, which, like the nitrate of albumen before mentioned, is not coagulable by heat; the albumen, therefore, remains in solution, and is not detected.

I cannot too emphatically insist upon the fact that the smallest trace of albumen in the urine, whether with or without the appearance of renal

tube-casts, is always abnormal and pathological. Not long since, during a discussion at the Clinical Society, it was suggested by one speaker that an amount of albumen so minute as to require for its detection any test more delicate than heat and nitric acid should be looked upon as physiological. I scarcely need say that such a distinction would be purely artificial, and not in accordance with facts.

The *amount* of albumen present is, for clinical purposes, sufficiently expressed by the terms 'opalescence' for the slightest degree of coagulation, 'milkiness' for a greater degree of turbidity, and when the amount of albumen is still greater, the proportion which the coagulated sediment bears to the column of urine, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{10}$ &c., is sufficiently indicative of the degree of albuminous impregnation, and conveys a more definite idea than a statement of the percentage of dried albumen. If a liquid test has been used, as, for example, the solution of picric acid, allowance must of course be made for the extent to which the urine has been thus diluted.

I said just now that picric acid, as a test for albumen, may be used in the form of powder or crystals. In this form it may conveniently be car-

ried in the pocket for bedside use. Mr. Hawksley, of 357 Oxford Street, has made for me a waist-coat-pocket test-case, which consists of a test-tube 4 inches in length, in which are packed two smaller corked tubes 2½ inches in length, one containing powdered picric acid, the other grain lumps of caustic potash for sugar testing. The outer tube is enclosed in a leather case. In another similar case may be carried a small nipple pipette, which is very convenient for transferring urine or water to the test-tube. My friend Mr. Casella, 247 Holborn Bars, has made for me very neat metal cases to contain the above-described apparatus ; also a metal case to contain a small pocket spirit-lamp.

In testing with the powder, about one-third of a grain, equal in bulk to a peppercorn, may be shaken up in a test-tube, with a column of urine about one inch in height. As the powder dissolves, the urine becomes turbid with coagulated albumen. The object is to add as much of the powder as the urine will dissolve, and no more. In the absence of a test-tube, the powder may be stirred up with the urine in a wine-glass. The solution of the picric acid in urine, and the coagulation of the albumen, are quickened by heating

the tube over a spirit-lamp or candle, or by immersing the tube or glass in hot water.

The dry picric acid should be used in the form of fine powder, not in crystals. One of my correspondents was perplexed by finding that crystals of the acid dropped into a highly albuminous urine became surrounded by a film of coagulated albumen, and, becoming caked together, formed a dense precipitate. This can never occur if the fine powder be shaken up with the urine.

Another convenient mode of using the powder at the bedside is to add twenty or thirty minims of water to the peppercorn bulk of the acid in a tube, and quicken the solution by the application of heat; an equal bulk of urine is then gradually added to the hot saturated solution, when albumen, if present, is at once detected. It has been objected to the picric acid solution that it stains the fingers; but this stain, unlike the yellow stain caused by nitric acid, which penetrates into the skin, and remains for several days, is merely a surface-stain, and is immediately and completely removed by washing with soap and water.

Dr. George Oliver[1] has devoted much time and

[1] *On Bedside Urinary Testing.*

labour to a comparison of the various tests for albumen, and also to the preparation of test-papers for bedside use. His observations agree with my own, that the three most delicate liquid tests for albumen are solutions of acidulated potassio-mercuric iodide, picric acid, and tungstate of soda. He finds 'these three albumen-precipitants of equal keenness;' but I have found the tungstate of soda decidedly inferior to the other two. The potassio-mercuric iodide solution and picric acid in saturated solution I find of equal delicacy; but I also find that when urine contains only a minute trace of albumen the picric acid *powder* dissolved in the boiling urine gives a more decided opalescence than the acidulated potassio-mercuric iodide solution. In the form of paper-test, Dr. Oliver states that picric acid is 'the weakest of the series' (*Lancet*, Jan. 27th and Feb. 8th, 1883). The explanation of this result is that, in consequence of the comparative insolubility of picric acid, the small slips of paper dried after immersion in a saturated solution do not retain sufficient of the acid to render them a satisfactory means of testing. But there is no need for picric acid *papers*. The main advantage of the test-papers consists in their ready portability;

but this advantage is shared equally by the picric acid powder, which, moreover, possesses these advantages over any forms of test-papers—that it requires no elaborate preparation or the addition of any other agent, such as acetic or citric acid ; that it is therefore cheaper and more simple ; that it affords a more ready and trustworthy indication of the amount of albumen ; and, lastly, as I shall show you in a future lecture, that, in combination with caustic potash, it forms an admirable qualitative and quantitative test for sugar.[1]

[1] I was induced to use picric acid as a test for albumen by a suggestion from my son, G. Stillingfleet Johnson, who found, as he states in a paper on the Compounds of Albumen with Acids, published in the *Journal of the Chemical Society*, August 1874, that picric acid caused coagulation of albumen in solutions of all the acid compounds of that substance. It was not until after I had (in the *Lancet* Nov. 4, 1882, p. 737) published my experience of picric acid as an albumen test that I became aware of the fact that the same test, although known to very few, had for some years been occasionally used by others—see my letter in the *Lancet* (Nov. 11, p. 823). As will be found stated in the following Lecture, my first observation of the reaction of *glucose* in a boiling solution of picric acid and caustic potash was the result of what I have ventured to call 'a happy accident.'

LECTURE II.

ON THE VARIOUS MODES OF TESTING FOR SUGAR IN THE URINE.

GENTLEMEN,—In my previous lecture I described and demonstrated the various modes of testing for albumen in the urine; on the present occasion I propose to show you the chief tests for sugar, and to estimate the relative value of each. I will first show you what is called Moore's test. I pour about a drachm of saccharine urine into a test-tube, and add half its bulk of liquor potassæ, then heat the mixture over the lamp; and after boiling it for a minute or two, you see it gradually assume a brandy-brown colour. This test is easily applied, but it is not very delicate, since it will not indicate a proportion of sugar less than about two grains to the ounce. I will presently show you far more delicate tests. Then Moore's test has sometimes misled inexperienced observers in this way:—Liquor potassæ

often contains lead from the bottles in which it has been kept, and when lead-contaminated liquor potassæ is boiled with albuminous urine, the sulphur of the albumen combines with the lead to form a dark sulphide, which unpractised observers might mistake for the brown colour produced in saccharine urine.

The Fermentation-Test.—When saccharine urine is mixed with yeast and kept warm, fermentation takes place with the evolution of carbonic acid and the formation of alcohol. Dr. Wm. Roberts (on *Urinary and Renal Diseases*) has shown that this test may be made use of for a quantitative analysis. As the sugar is decomposed, the specific gravity of the urine falls. Each degree of specific gravity lost indicates one grain of sugar to the ounce. The great objection to this method is the length of time, at least twenty-four hours, required for its completion. The urine is capable of absorbing about its own bulk of carbonic acid, and, according to Dr. Roberts, urine containing two and a half grains to the ounce or less, gives no visible sign of fermentation. It is therefore less sensitive than even Moore's test.

Trommer's Test.—To this saccharine urine in a

test-tube I add a drop or two of a solution of sulphate of copper, and to this an excess of liquor potassæ. The oxide of copper, which is first thrown down, is redissolved, and forms a clear blue liquid. Now, on applying heat the oxide of copper is reduced to a suboxide, which forms a dense red or yellow deposit. An excess of copper, not being dissolved, may cause confusion, and the dark brown colour, from the action of the potash on the sugar, may interfere with the result.

Fehling's Solution.—A more satisfactory mode of applying the copper-test is in the form of Fehling's solution, which contains the following ingredients:—sulphate of copper 90½ grains, neutral tartrate of potash 364 grains, solution of caustic soda, specific gravity 1·12, four fluid ounces, cold water to make up six fluid ounces. To use this solution, a column of about three-quarters of an inch is poured into a test-tube, and heated until it boils, and then a drop or two of the urine to be tested is added. In a few seconds, if the urine contain much sugar, the liquid becomes of an opaque yellow colour, and a copious red or yellow precipitate falls. If the quantity of sugar be small, the urine is added more freely, but not beyond the

volume of the copper-solution. Fehling's solution soon undergoes change by keeping, a portion of the oxide of copper becomes precipitated, and then, of course, the strength of the test is changed. When the test has been kept for some time, it will deposit suboxide on boiling without the presence of sugar. This is one reason for boiling the test before adding the suspected urine. If boiling the liquid render the test turbid, it must be filtered, or a fresh solution prepared. When the solution is used for a quantitative analysis, it must be freshly prepared, and its strength is such that 100 minims are reduced by half a grain of sugar, the complete reduction being shown by the decoloration of the liquid, and the precipitation of the suboxide of copper. When the urine is highly saccharine, it must be diluted to a definite proportion, five, ten, or more times, before the analysis is made, and then the result is to be multiplied by the number of dilutions.

Dr. Pavy has modified and improved upon Fehling's method of analysis.[1] The essential difference between the method of Dr. Pavy and that of Fehling consists in the addition of a sufficient

[1] *Lancet*, March 1, 1884, p. 376.

quantity of *ammonia* to the copper-solution to prevent the precipitation of the cuprous oxide, after its production by the reducing action of the glucose. Rochelle salt (potassic tartrate of soda), though it effectually dissolves cupric oxide, is incapable of dissolving cuprous oxide, and some difficulty is often experienced by Fehling's process in ascertaining the exact moment of disappearance of the blue colour due to cupric copper, on account of the turbidity and red tint imparted by the precipitated cuprous oxide. This difficulty is removed by Dr. Pavy's process, since the ammonia altogether prevents the precipitation of cuprous oxide, and in a clear solution the exact amount of sugar required to completely decolorise the cupric blue tint may be much more easily determined. The chief precaution necessary is to completely exclude air during the determination, because a colourless ammoniacal cuprous solution is rapidly rendered blue by exposure to atmospheric oxygen, the cupric hydrate being thereby reproduced.

The Picric Acid and Potash Test for Sugar.—In a letter which I published in the *Lancet*, November 18th, 1882, I stated that I had accidentally stumbled upon the fact that picric acid, when

boiled with caustic potash, forms a most delicate test for glucose. I added some picric acid solution to a boiling specimen of diabetic urine, which had been previously mixed with liquor potassæ, and found, to my surprise, that the liquid at once assumed an intensely dark colour. I was not then aware of the fact that this reaction of picric acid with grape-sugar had been observed by Braun, a German chemist, nearly twenty years ago ('Ueber die Umwandlung der Pikrinsäure in Pikramminsäure, und über die Nachweisung der Trauben-Zucker.' C. D. Braun, *Zeitschrift für Chemie*, 1865). In this paper, it is shown that grape-sugar, when boiled with picric acid and potash, reduces the yellow picric acid to the deep red picramic acid, the depth of colour depending on the amount of sugar present. I am not aware that hitherto any attempt has been made to utilise this as a qualitative clinical test for sugar in the urine, or as a means of accurately estimating the amount of sugar in a saccharine solution. I think, however, that, after having been the subject of much hostile criticism, the value of the test for both purposes has been completely proved and established.

I take a fluid drachm of a solution of grape-

sugar, in the proportion of a grain to the fluid ounce, mix it with half a drachm of liquor potassæ (*P.B.*), and forty minims of a saturated solution of picric acid, and make up the mixture to four drachms with distilled water.[1] The mixture is conveniently made in a boiling tube ten inches long and three-fourths of an inch in diameter, which

[1] We have ascertained by careful experiment that, while half a drachm of liquor potassæ may be used as a constant quantity for the analysis of saccharine solutions of any strength, the proportion of picric acid solution should not be less than forty minims, even for the analysis of weak solutions of glucose. If a grain to the ounce solution be boiled with a less proportion of picric acid, the resulting colour is slightly, though appreciably, paler than when the full amount of the acid is used. When the full proportion of picric acid is added, a yellowish precipitate of picrate of potash falls in the cold liquid. This is redissolved before the liquid reaches the boiling point. That the deeper tint obtained by boiling a drachm of solution of glucose, containing *one grain* of glucose per fluid ounce with thirty minims of liq. potassæ and forty minims of picric acid solution, and water up to four drachms, is not due to any red coloration produced by the action of the potash upon the excess of picric acid present, is proved by the fact that the colour thus obtained is identical with that which is observed when one drachm of solution of glucose, containing *four grains* of glucose per one fluid ounce, is boiled with thirty minims of liq. potassæ, forty minims of picric acid solution, and water up to four drachms, and the resulting dark liquid is subsequently *diluted four times*. In this latter case, the excess of picric acid present over and above the quantity necessary for complete interaction between it and the total sugar in solution is very small. Either of the above processes therefore might be employed for fixing the quarter-grain standard.

should be marked at the height of four drachms. With a long boiling tube, there is little risk of the liquid boiling over; and the steam, condensing in the upper cooler part of the tube, flows back as liquid, so that there is little loss by evaporation. The liquid is now raised to the boiling point, and kept boiling for sixty seconds, so as to ensure complete reaction between the sugar and the picric acid. During the process of boiling, the pale yellow colour of the liquid is changed to a beautiful claret-red.

The liquid having been cooled by cautiously immersing the tube in cold water, we ascertain that its level is that of the four-drachm mark on the tube; and if found below the mark, it is brought up to it by the addition of distilled water. The colour now is that which results from the decomposition of picric acid by a grain of sugar to the ounce four times diluted; in other words, it indicates one quarter of a grain of sugar to the ounce, and this colour is a convenient standard for comparison in making a quantitative analysis. The picramic acid solution, however, on exposure to the light even for a few hours, becomes paler; but the colour may be exactly imitated by a solution of ferric acetate with an excess of ferric perchloride, and a slight excess of acetic acid. The following

is the formula for the standard solution, for which, and for much other valuable aid in working out the details of the analytical process, I am indebted to my son, G. Stillingfleet Johnson, F.C.S.

Liq. ferri perchlor. fort. (sp. gr. 1·338), ʒj ; liq. ammon. acet. (sp. gr. 1·017), ʒiv ; acid. acet. glacial. (sp. gr. 1·065), ʒiv ; liquor ammoniæ (sp. gr. 0·959), ʒj ; aquæ destil. ad ℥iv.[1]

Mix thoroughly the iron solution with the liq. ammon. acet. and the acetic acid, then add the liq. ammoniæ, and dilute up to four ounces. The ingredients are all of the strength prescribed by the British Pharmacopœia.

The colour of this is equal to a quarter of a grain of grape-sugar to the ounce. When a fresh solution is made, it should be checked by comparison with a grain-solution of sugar, boiled with picric acid and potash, and four times diluted as above described.

I have here an iron standard solution, which was made six months ago, and which, having been kept for the most part in the dark, retains its colour unchanged. I have also a solution of grape-

[1] This standard solution may be obtained from Messrs. Bell and Co., 225 Oxford Street, from Messrs. Savory and Moore, 143 New Bond Street, and probably from other pharmaceutical chemists.

sugar, one grain to the ounce, in eighty per cent. of water and twenty per cent. of rectified spirit, which has kept without change in an accurately stoppered bottle for the same period. The alcohol prevents the spontaneous decomposition of the sugar, but has no reducing action on picric acid.

If, now, a drachm of a solution of grape-sugar, containing *two* grains to the ounce, be mixed with the same quantity of liquor potassæ and picric acid, and made up to four drachms in the boiling tube, the result of boiling the mixture as before for sixty seconds will be the production of a much darker colour than when the one-grain solution was acted upon; but, if now the dark liquid be diluted with its own volume of water, the colour will be the same as that of the one-grain solution.

The dilution is accurately done in a stoppered tube, twelve inches long and three-quarters of an inch in diameter, graduated into 10 and 100 equal divisions. By the side of this tube, and held in position by an S-shaped band of metal, is a stoppered tube of equal diameter, and about six inches long, containing the standard iron-solution.[1] (See Fig.)

[1] This picro-saccharometer and the other apparatus required for a quantitative analysis are made by E. Cetti, 36 Brooke Street, Holborn, E.C.

Sufficient of the dark saccharine liquid to be analysed is poured in to occupy exactly ten divi-

The picro-saccharometer, as described in the text.

The shading of the side-tube indicates the ferric acetate standard. The darker shading at the bottom of the graduated tube shows the saccharine fluid, darkened by boiling with picric acid and potash, and occupying ten divisions before dilution.

sions of the graduated tube. Distilled water is then added cautiously, until the colour approaches that

of the standard. The level of the liquid is then read off and noted. A more exact comparison of the saccharine liquid with the standard is made by pouring into a flat-bottomed colourless tube, about six inches long and an inch in diameter, as much of the standard as will form a column of liquid about an inch in height, and an exactly equal column of the saccharine liquid in a precisely similar tube. The operator then looks down through both tubes at once, one being held in each hand, upon the surface of a white porcelain slab, or a piece of white paper. In this way, a slight difference of tint is readily recognised; and, if the liquid to be analysed be found to be darker than the standard, it is returned to the saccharometer and diluted until the two liquids are found to be identical in colour, when the final reading is taken. If we are in doubt whether the saccharine liquid is darker than the standard, a few drops may be poured back into the saccharometer, so as slightly to shorten the column of liquid in the test-tube; and if now the tints appear more nearly equal, it is evident that the liquid requires rather more dilution. The saccharine liquid having been diluted four times before it was boiled, a colour equal to that of the quarter-grain standard would indicate

one grain of sugar per fluid ounce. If further dilution were required—say from ten to twenty divisions—the proportion of sugar would be two grains per ounce, and so on to thirty or forty or upwards, or to intermediate divisions. Thus, dilution from ten to thirty-five divisions would indicate 3·5 grains of sugar per ounce.

We have found, by experiment, that ten minims of a cold saturated solution of picric acid are rather more than sufficient for decomposition by one drachm of a solution of grape-sugar in the proportion of one grain to the ounce. A drachm of the solution would of course contain one-eighth of a grain of sugar. In making an analysis, the picric acid must be added in proportion to the amount of sugar. If the proportion of sugar be as high as six grains per ounce, a drachm of the picric acid solution will be required. If the proportion of sugar be higher than this, the saccharine fluid should be diluted with distilled water, in a definite proportion, before commencing the analysis, and the product of the analysis of the diluted fluid is then to be multiplied by the degree of dilution—two, five, or ten, as the case may be, to which the original fluid has been subjected.

When the urine has been diluted ten times, the figures on the saccharometer indicate the number of grains per ounce. Thus, when the ten times diluted urine, after boiling with picric acid and potash, is further diluted from 10 divisions to 35, to obtain the standard colour, the amount of sugar is 35 grains to the ounce.

To reduce the amount of sugar per ounce to the proportion per cent., we have to remember that an ounce of water at 0° Cent. weighs 437·5 grains. If now the proportion of sugar is 40 grains per ounce, then as 437·5 : 100 :: 40 : 8·19.

Distilled water, or clear rain-water, should be used for diluting. Hard water, containing salts of lime, is rendered turbid by the carbonate of lime precipitated by mixture with caustic potash, and any turbidity in the liquid interferes with the exact estimation of the depth of colour. In testing undiluted urine, a slight turbidity often results from separation of phosphates by the potash. This turbidity may be removed by allowing the phosphates to form a sediment, or more speedily by filtration. When a highly saccharine liquid is diluted five or ten times before mixture with the testing materials, no phosphatic turbidity occurs.

In making a volumetric analysis, care must, of course, be taken that the measurements and dilutions are accurately made.

The preliminary dilution of a strongly saccharine specimen may be made in the graduated saccharometer tube; or, into a flask graduated to contain fifty cubic centimètres, five or ten cubic centimètres of the saccharine liquid may be delivered from a graduated measure or pipette;[1] then, the flask being filled up to the graduation with distilled or soft water, the dilution will be ten times with five cubic centimètres, and five times with ten cubic centimètres of the liquid to be analysed. Or, without any special apparatus, an accurately measured drachm of urine may be diluted up to five or ten drachms with distilled water.

Another important point is that, while the amount of potash remains the same, the picric acid must be in proportion to the amount of sugar in solution. It has already been mentioned that ten minims of the picric acid solution are more than equal to one-eighth of a grain of glucose, which is

[1] If a graduated *measure* is used it should be rinsed out after pouring the urine from it into the diluting flask with some of the water used for diluting, but a *pipette* is graduated to deliver a certain measure of liquid without subsequent rinsing.

the amount contained in one drachm of a solution, in the proportion of a grain to a fluid ounce. A considerable excess of picric acid does not appreciably affect the colour of the picramic acid, while a deficiency would, of course, lead to an underestimate of the amount of sugar. If an analysis with forty minims of picric acid solution indicate, say, from four to five grains of sugar, it is probable that some sugar has been left undecomposed ; and a second analysis, with a larger proportion of picric acid, might therefore give a higher and a more correct result. If, on the other hand, a second analysis, with a larger proportion of picric acid, give an identical result, we may feel certain that the whole of the sugar has been decomposed, and the amount correctly indicated by the resulting picramic coloration. In any case, when the amount of sugar indicated is less than would suffice to react upon the amount of picric acid employed, the result may be relied upon as correct.

The presence of albumen, even in large amount, has but little influence on the picric acid test for sugar. In illustration of this, the following experiments will suffice. A specimen of urine, normal as regards the amount of saccharine or saccharoid

material, but containing a large amount of albumen, was boiled with picric acid and potash, with sufficient water to dilute the urine by its own volume of liquid. A second portion was treated in the same way after the separation of the albumen by boiling and filtration, and the first specimen gave a darker tint than the second to a degree that might be considered to indicate one-tenth of a grain of sugar per ounce. Another portion of the urine was decolorised by repeated filtering through charcoal; and, of this, one specimen was tested while it retained its albumen, another after the separation of the albumen; the result being that both yielded identical tints of colour, and this was very slightly paler than that of the specimen which was tested after having been deprived of its albumen without previous decolorisation by charcoal. The explanation is, that pure albumen has no reducing influence on picric acid when boiled with dilute potash, such as is used in testing for sugar; but with seralbumen, as with white of egg, there is associated a colouring matter which is partly separated by filtering off the coagulated albumen, and entirely removed by repeated filtering through charcoal. The colouring matter in question has a slight reducing influence on picric

acid, although the colouring matter of normal urine has been found to have none. The coagulated albumen collected on the filter, after being thoroughly washed, gives no red reaction when boiled with picric acid and potash diluted in the same proportion as that employed in testing for sugar. This has been proved by repeated experiments.

When I first published to the profession my observations on picric acid and potash as a test for sugar, it was suggested that unoxidised sulphur-compounds in the urine, and especially in albuminous urine, would form an alkaline sulphide when boiled with potash, and this would decompose the picric acid and render the test fallacious. Experience has proved that these theoretical objections were quite groundless. My son proved conclusively that, when pure albumen is boiled with diluted solutions of potash, such as are used in testing for sugar, no alkaline sulphide is formed.

The final communication of my son, in which he demonstrates that the apparently contradictory results obtained by different observers are explained by the varying proportions of the caustic potash employed, is published in the *Chemical News*, February 23, 1883, page 87.

The accuracy of the picric acid method of volumetric sugar-analysis has been fully and fairly tested by my son and myself. Our plan has been to compare the results of this process with those obtained by Dr. Pavy's ammonio-cupric method. We have analysed the same specimens, many of them albuminous as well as saccharine, by the two processes; and our results are found to be practically identical, the differences being only such as are due to unavoidable slight errors in conducting an experiment. Both methods, in fact, are based upon the same chemical principle—namely, that glucose, when heated with potash in the presence of an oxidising agent, has a tendency to rob it of its oxygen. In the one process, the reducing action of the sugar is exerted upon an oxide of copper; in the other, on picric acid. A definite weight of sugar reduces, in the one case, a proportional amount of cupric oxide, and in the other an equivalent proportion of picric acid, with resulting picramic acid and a corresponding measurable intensity of colour. In analysing pure solutions of glucose in water the two methods give identical results, but in the majority of cases of urinary analysis the ammonio-cupric process gives results

slightly in excess of the picric acid method. This excess is due to some non-saccharine ingredients in the urine, which reduce cupric oxide, but not picric acid. Amongst other ingredients of normal urine, uric acid and urates are known to have this reducing effect on cupric oxide.

I claim for the picric acid and potash method of analysis that it is as accurate as any other, and that for the estimation of sugar in the urine it is even more accurate than either Fehling's or Dr. Pavy's process, because the picric acid is not acted on by uric acid or urates, which reduce the oxide of copper. The method of analysis by the picro-saccharometer is more speedy and more easily acquired than any other. The materials and apparatus required are easily prepared, inexpensive, and not, like Fehling's copper-solution, liable to undergo rapid changes. For exact results with the picric acid process, the main requisite is that the standard should be accurate. This is as important as the exact proportion of copper in Fehling's or Pavy's volumetric solution. The standard iron-solution, as I have shown, may be kept in the dark for months without the slightest change of colour; and a solution of grape-sugar in

water, one grain to the ounce, with twenty per cent. by volume of rectified spirit, may be kept unchanged for an indefinite period, and used occasionally for comparison with the ferric acetate standard. For obtaining a solution of glucose of known strength we have relied entirely upon Dr. Pavy's ammonio-cupric process.

During the last nine months, I have tested with the picric acid and potash a large number of specimens of *normal* urine, with the almost uniform result of a depth of colour indicating the proportion of 0·6 grain of sugar in the fluid ounce, the indication usually varying between the limits 0·5 and 0·7 grain in the fluid ounce. In a considerable number of cases we have tested the same specimens by the ammonio-cupric method, with the indication usually of from 0·7 grain to 0·9 grain in the fluid ounce; *i.e.*, an excess of that obtained by picric acid of from 0·1 to 0·3 grain in the fluid ounce.

The following have been the proportions of the various liquids: a drachm of urine, ½ a drachm of liquor potassæ, 40 minims of picric acid solution, made up to 2 drachms with distilled water. The mixture is kept boiling for a minute,, and, when cooled, is compared with the standard. The urine

having been diluted by its own volume, a depth of colour equal to that of the standard would indicate 0·5 grain of sugar; but, in nearly every case, I have found it so much darker than the standard, as to require further dilution equal to 0·1 grain before the standard colour is reached, thus giving an indication of 0·6 grain.

When the mixture is rendered turbid by phosphates, these must be removed by filtration before the colour can be quite accurately estimated.

So constant is this degree of coloration with normal urine that if, instead of diluting up to 2 drachms, the dilution be carried further by 24 minims, the resulting colour might be taken as an approximation to an accurate quarter-grain standard, and, in the absence of a more exact standard, might be used for making a quantitative analysis. The question arises: Does normal urine contain as much as 0·6 to 0·7 grain of glucose in the fluid ounce? I am not prepared to assert this without further evidence than we have as yet been able to obtain; but, if it be not glucose which gives these almost identical analytical results with the two processes, it must surely be some nearly allied substance.

One difference between normal urine and weak solution of glucose consists in the fact that while the former gives some red coloration with picric acid and potash while cold, this coloration being increased by boiling, a pure solution of glucose or a highly saccharine urine gives no red colour until the mixture of the urine with the reagents has nearly reached the boiling point. Fehling's solution is decolorised by boiling with normal urine, but a precipitate of suboxide is prevented by some constituents of the urine which keep it in solution. With reference to this subject Dr. Beale relates the following instructive experiment. A precipitate of suboxide of copper was obtained by boiling a solution of grape-sugar with alkaline copper solution. To a portion of suboxide produced in this way about a drachm of healthy urine was added, and the reddish precipitate was instantly dissolved, forming a perfectly clear solution. (Kidney Diseases, Urinary Deposits, &c., p. 246.) I have repeated this experiment with the same result.

Since using the picric acid and potash test for sugar I have so often found an excess of sugar in urine when from the comparatively low specific gravity I should not have suspected it, that I

now test every specimen of urine first for albumen and then for sugar by the following simple, rapid, and trustworthy method. To about a drachm of urine I add its own volume of picric acid solution. If the liquid remain clear no albumen is present. If a precipitate occurs, not dissolved by boiling, there is albumen in proportion to the amount of precipitate. I now add half a drachm of liquor potassæ and boil for a few seconds; the coagulated albumen if present is dissolved by the alkali and a red or black coloration occurs. If, when an ordinary half-inch test-tube is held up to the light, a red colour is visible through the liquid, there is no more than the normal amount of saccharine matter, less than a grain to the ounce. As little as two grains to the ounce will render the liquid inky black so that no light is transmitted through the tube. The amount of sugar must then be determined by the quantitative method.

For bedside sugar-testing I carry in my pocket case before described (p. 19), in addition to powdered picric acid, grain lumps of caustic potash, and a test-tube which is graduated up to 3 drachms. I put into the test-tube about a third of a grain of picric acid, as much as can be

carried on the point of a penknife; then with the nipple pipette, or without it, half a drachm of water. The acid is dissolved in the water by the heat of a lamp or candle; now half a drachm of urine is added, and the presence or absence of albumen is ascertained; next a grain lump of caustic potash is added, and the liquid is boiled for a few seconds. In normal urine the resulting colour will be somewhat darker than the ¼-grain standard until dilution with water is carried up to the twelve-minim mark above the drachm, indicating 0·6 grain per ounce. If the colour be still darker, more water is added. If dilution to two drachms gives the ¼-grain standard colour there is one grain to the ounce, the urine having been diluted from ½ drachm to 2 drachms; dilution to the three-drachm mark would indicate 1½ grain per ounce. If the colour is still darker than the standard, the urine must be diluted before it is tested. This may be done by means of the nipple pipette, which is graduated from 5 minims up to 30 minims. Thus 5 or 10 minims of urine may be diluted up to 10 or 20 or more times the volume with water. The diluted liquid is then tested in the small graduated tube, and the result multiplied by the number of

dilutions. If after boiling a ten times diluted urine, as directed, with picric acid and potash, the standard colour is reached by dilution to the one-drachm mark, there will be 5 grains of sugar per ounce. If dilution up to two drachms is required, the amount will be 10 grains; to the three-drachm mark 15 grains, and for the intermediate dilutions the amount may be estimated with a near approach to accuracy.

The Indigo-Carmine Test.—Dr. Oliver has recently had papers prepared by drying after immersion in a solution of indigo-carmine with carbonate of soda. A paper is placed in a test-tube and covered with distilled water; heat is then applied until a blue solution is formed; a drop of diabetic urine is then added, and the heating is continued. The solution changes from blue to violet, purple-red, yellow, and finally straw colour. After cooling and exposure to the air, the liquid passes back through the various colours to the original blue. The delicacy of the test is increased by using a carbonate of soda paper with the indigo-carmine paper.

Polarising Saccharometers.—I will not attempt to show you or describe to you the various forms

of Polarising Saccharometers, for they appear to me to be too complicated and most of them too costly for ordinary use.

And now, in conclusion, let me impress upon you the importance of testing the urine for both albumen and sugar in all cases of disease that come under your observation. You know that the urine of every patient admitted into this hospital is thus tested; and until this practice becomes general throughout the profession, many cases of essentially curable albuminuria and glycosuria will be overlooked until they have reached an incurable stage.

Of the practical importance of testing for albumen I have already said enough in my first lecture; and now I propose to add a few practical remarks on the subject of glycosuria. And in the first place you will have to bear in mind that the terms glycosuria and diabetes are by no means synonymous. All urines, as we have seen, contain a fraction of a grain of saccharine matter which neither the picric nor the copper test can distinguish from glucose; but beyond this quite normal condition of urine we not infrequently meet with glycosuria in proportions varying from three or four grains to fifteen or twenty grains of glucose per ounce, the

urine being normal in quantity and specific gravity, and there being no complaint of thirst or other symptom of diabetes. In a large proportion of cases these patients have passed middle age; they are more or less troubled by flatulence, irregularity of the bowels, and other symptoms of dyspepsia. Questioned as to their dietary, they confess to a fondness for various forms of saccharine food; and, in short, these are cases in which the taste for such food has survived the power of digesting it. The result is that undigested sugar is eliminated, and a rigid abstinence from saccharine food, including of course all kinds of fruits, is often followed by the speedy and complete disappearance of the glycosuria. These curable cases of saccharine dyspepsia in middle-aged and elderly people differ essentially from the intractable diabetes which is so often met with in young subjects.

The following is a typical example of this form of saccharine dyspepsia. On December 8th I was consulted by an oculist in large practice in the north of England. He was forty-nine years of age, working hard, living well, and not taking much exercise. He had had gout more than once. Eight days ago he began to be giddy with confusion of

thought, and a physician whom he consulted found a trace of albumen in his urine. I found the urine with a sp. gr. 1021, a trace of albumen, at once shown by picric acid and slowly by nitric acid. Then boiled with picric acid and potash, it became inky black, and the saccharometer indicated six grains of sugar per ounce. There were no diabetic symptoms, but he was manifestly dyspeptic and confessed to a great liking for sweets. These of course I advised him to discontinue, and I prescribed a mixture with quinine, strychnia, hydrochloric acid, and ginger. On January 25, when I again saw him, the albumen had gone and the sugar was reduced to one grain per ounce. An eczematous rash which had troubled him for some months had disappeared. In this case, as in many cases of glycosuria, the albuminuria was probably a result of irritation of the kidneys by the unassimilated sugar, and the skin eruption was probably due to the same cause.

This is one of several cases which have lately come under my observation, in which the routine practice of applying the picric acid and potash test for sugar has led to the unexpected discovery of variable but excessive amounts of glucose, and

has thus given a clue to the successful treatment of the associated symptoms.

In conclusion let me briefly recapitulate the main points regarding the tests which have hitherto been commonly employed for the detection of sugar in urine. These tests are :—

1. Moore's Test.
2. The Fermentation Test.
3. Trommer's Test.
4. Fehling's Test.
5. Pavy's Ammonio-Cupric Method.

1. *Moore's Test* is easy of application, and is not affected by any substances usually present in urine except glucose. Its disadvantages are its want of delicacy, and the fallacy which may result from the presence of lead in the liquor potassæ (p. 23).

2. *Fermentation Test.*—There are fallacies connected with this test which have been fully described (p. 24), and it is also objectionable on account of the length of time required for its completion.

3. *Trommer's Test.*—This is a good qualitative test, but as it cannot detect less than two grains of glucose in a fluid ounce of urine, the negative evidence which it may afford is of comparatively little value (p. 24).

4 & 5. The chief objections to *Fehling's* and *Pavy's* tests for the quantitative estimation of sugar in urine are 1st. that they are affected by uric acid, which is a constant ingredient of that secretion. For clinical purposes the difference of a fraction of a grain per ounce is of no practical importance, but 2nd., the successful working of both these methods requires an amount of manipulative skill which is not easily acquired without special instruction in a laboratory. Dr. Pavy's Ammonio-cupric process, admirably suited as it is for the laboratory, has the special disadvantage for the consulting-room that during its performance the air of the room becomes unpleasantly charged with ammoniacal vapour.

Those who use this test will be grateful to Dr. Pavy for the elaborate table which he has published ('Lancet,' March 1, 1884) to facilitate the calculation of the amount of sugar in a given solution.

Finally, the picric acid method possesses all the advantages of the before-mentioned tests, whilst it is free from their attendant drawbacks. Thus as a qualitative test it is more rapidly completed and more delicate than Moore's and Trommer's tests, and indicates the smallest increase of the normal amount of saccharine matter in the urine. And as

a quantitative method it is free from the objection which applies to all the copper tests, of being affected by uric acid or other normal ingredients of the urine; neither does the presence of albumen interfere with the action of the test, as it does with all the forms of copper testing. Then as regards accuracy and facility of operation, having worked diligently at all the methods, I am in a position to make a comparison, and I have found it easier to distinguish between slight shades of red colour in working the picric acid process than to be assured of the exact period of complete disappearance of the blue colour in the copper solution, even when using Dr. Pavy's improved ammonio-cupric test.

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